

GNE.3238

PATENT

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant : Goddard, et al.  
Appl. No. : 10/063,566  
Filed : May 2, 2002  
For : SECRETED AND  
TRANSMEMBRANE  
POLYPEPTIDES AND NUCLEIC  
ACIDS ENCODING THE SAME  
Examiner : Hunnicutt, Rachel Kapust  
Group Art Unit : 1647

**DECLARATION UNDER 37 CFR §1.131**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Dear Sir:

We declare and state as follows:

1. We are the inventors of the invention claimed in the above-captioned patent application.
2. During the time period in which all of the events and activities described herein occurred, we were employed by Genentech, Inc., the assignee of the above-captioned application.
3. All of the events and activities described herein were performed by us personally, or by others at our direction as part of our duties as employees of Genentech, Inc.
4. The invention claimed in the above-captioned patent application was conceived prior to April 29, 1998 and diligently reduced to practice thereafter in the U.S. as described below.
5. Prior to April 29, 1998, we conceived of the invention claimed in the above-captioned patent application. Prior to April 29, 1998, the idea of investigating several newly discovered DNA sequences for their relevance, including developing primers and cloning the DNA sequences of interest from normal and tumor tissues, was conceived. The attached sequence printout (Exhibit A), dated prior to April 29, 1998, shows the complete sequence of the nucleic acid having the sequence of SEQ ID NO:57, as well as the complete sequence of the amino acid of SEQ ID NO:58. Thus, conception of the invention claimed in the above-captioned patent application occurred prior to April 29, 1998.

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6. The date deleted from Exhibit A is prior to April 29, 1998. This date was redacted pursuant to M.P.E.P. § 715.07. The date that remains is the date the report was printed, December 21, 2004.

7. After these initial experiments, we continued to produce primers, clone and sequence other DNA sequences. We then began to identify the expression levels of the cloned sequences, and created constructs for expression of the encoded proteins. The enclosed printed copy of an electronic file (Exhibit B) shows that PCR primers for numerous sequences which had been previously identified were designed on March 6, 2000 (as evidenced by the file name, "oli.out3\_6\_00"), including the primers for DNA59609, the DNA which is relevant to the presently claimed invention (see top of page 3 of Exhibit B). This data shows diligence in reducing to practice following conception of the invention. Thereafter, the primers were tested for expression in various normal and tumor tissues on June 13, 2000 (Exhibit C). These pages show a series of gels and a summary of results in which the primers were used to determine tissue and tumor expression levels for the various DNA sequences. The levels are shown using a ++, +, +-, and - to indicate the intensity of the specific signal which was detected. The data for DNA59609 is shown in row 10 on the results summary. This data shows that the DNA is more highly expressed in esophageal tumor as compared to normal esophagus tissue. Actual reduction to practice therefore occurred by at least June 13, 2000. Thus, we conceived of the present invention prior to April 29, 1998 and were diligent in reducing the invention to practice by at least June 13, 2000.

8. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

By: \_\_\_\_\_  
Andrey Goddard

Date: \_\_\_\_\_

By: \_\_\_\_\_  
Paul J. Godowski

Date: 2/15/05

By: \_\_\_\_\_  
J. Christopher Grimaldi

Date: \_\_\_\_\_

By: \_\_\_\_\_  
Austin L. Gurney

Date: \_\_\_\_\_

By: \_\_\_\_\_  
William I. Wood

Date: \_\_\_\_\_

Appl. No. : 10/063,566  
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By: Audrey Goddard  
Audrey Goddard

Date: Feb. 16/05

By: \_\_\_\_\_  
Paul J. Godowski

Date: \_\_\_\_\_

By: \_\_\_\_\_  
J. Christopher Grimaldi

Date: \_\_\_\_\_

By: \_\_\_\_\_  
Austin L. Gurney

Date: \_\_\_\_\_

By: \_\_\_\_\_  
William I. Wood

Date: \_\_\_\_\_

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By: \_\_\_\_\_  
Audrey Goddard

Date: \_\_\_\_\_

By: \_\_\_\_\_  
Paul J. Godowski

Date: \_\_\_\_\_

By: \_\_\_\_\_  
J. Christopher Grimaldi

Date: 2/15/2005

By: \_\_\_\_\_  
Austin L. Gurney

Date: \_\_\_\_\_

By: \_\_\_\_\_  
William I. Wood

Date: \_\_\_\_\_

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By: \_\_\_\_\_  
Audrey Goddard

Date: \_\_\_\_\_

By: \_\_\_\_\_  
Paul J. Godowski

Date: \_\_\_\_\_

By: \_\_\_\_\_  
J. Christopher Grimaldi

Date: \_\_\_\_\_

By: \_\_\_\_\_  
Austin L. Gurney

Date: 3/5/05

By: \_\_\_\_\_  
William I. Wood

Date: \_\_\_\_\_

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By: \_\_\_\_\_ Date: \_\_\_\_\_  
Audrey Goddard

By: \_\_\_\_\_ Date: \_\_\_\_\_  
Paul J. Godowski

By: \_\_\_\_\_ Date: \_\_\_\_\_  
J. Christopher Grimaldi

By: \_\_\_\_\_ Date: \_\_\_\_\_  
Austin L. Gurney

By: William I. Wood Date: 2/14/05  
William I. Wood

# EXHIBIT A

>Tuesday, December 21, 2004

>DNA59609 [Full]

>1380 Sites [All Sites]

DNA59609 goddarda GSeqEdit

>Sequenced by transposon tagging. ACGT Peter Ma and Ellison Chen.

```

tagl
sacI
sacI
hgAI/asphI[M.aluI-]
ecII361I
bspJ286[M.aluI-]
bsiHKA1
xhoI xhoI
tII1 tII1
tagl
xhoI mwoI bmyI
tII1 fnu4HI/dsofI mwoI xhoI
xhoI
tsp509I[M.ecoRI-] banII[M.aluI-] tII1
xhoI
tagl nspI bsh1236I
maeiI/hpyCH4IV bsiWJ/spI1
alul hlnII/acyI cac8I csp6I
sfiI sepi ahaII/bsaHI mluI snaBI hpy188I
tsp45I mboII aatII cac8I afIIII maeII/hpyCH4IV
maeiII earI/ksp632I hpy99I hpyCH4V bsaAI aluI apoI mwoI bsrBI auaI[M.tagI-] hlnII mwoI mmlI bts
1 ATGTGACACT ATAGAGAGAGC TATGACGTCC CATGACAGCG TACGTAAAGCT CGGAATTCCG CTCGAGCCGC TCGAGCTCGA GCCGAAATCGG CTCGAGGCGC
TACACTGTGA TATCTTCTCG ATTCTGCAGC GTACGTGCCC ATGCATTTCGA GCTTTAAAGCC GAAGTCGCCG AGCTCGAGCT CGGCTTAAGC GAAGTCCCGC
^start insert
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>Tuesday, December 21, 2004
>DNA59609 [Full]
>1380 Sites [All Sites]
>Sequenced by transposon tagging. ACGT Peter Ma and Ellison Chen.

```

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tagl
ssl1
sac1
hgiA)/aspH1[M.alu]-]
ec113611
bsp1286[M.alu]-]
bsiHKA1

thai
nlaIII xba1
sph1 fnuD1/mva1
nspH1 bslU1 tai1
tai1 nsp1 bsh12361
maei1/hpyCH41V bsiH1/sp11
alul hinf1/acy1 cac81 csp61
sfc1 sap1 aha11/bsaH1 mlu1 snab1 hpy1881
msp431 mbo11 aat11 cac81 a11111 mae11/hpyCH41V
maei11 ear1/asp6321 hpy991 hpyCH4V bsaH1 alu1 apo1 mwo1 bsrBI aua1[M.tag1-] hinf1 mwo1 mml1 bts

1 ATGTGACACCT ATTGAAGAC TATGACGTCG CATGACGCG TACGTAACT CGGAATTGCG CTCGAGCGGC TCGAGGCGC
TACACTGTGA TATCTTCTCG AACTGACAC GTACGTGCGC ATGCATTGCA GCTTAAACC GAGCTCGCG AGCTCGAGCT CGGCTTAAACC GAGCTTCCCG

*start insert

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n1a111          tai1 msp1          n1
hgi1a1/asph1  aci1 nspH1          mae11/hpyCH41V          rsa1          tag1 bsp
bsp1286        fnu4HI/bscf1        bsa1 hpa11          csp61 p1e1          bmy
bsi1KAI        hae111/pa11          rsa1 bsl1          sca1 mly1          ban
bmy1           cec81 nsp1           csp61 cfr101/bsrf1          bsr1          hinf1|M.taq1-
101 AGTGAAGAC CCAGCAGGCC GCCACATGC TCTGCTGTG CCTGATGATG CCGGTCATCG GGGAAAGCCCA GACCGAGTTC CAGTACTTTG AGTCGAAGG
TCACCTCGTG GGTGTCGG CGGTTGATCG AGACAGACAC GGACATGCAC GGCCAGTAGC CCCCTCGGGG CTGECTCAAG GTCAAGAAAC TCAGCTTCCC
1 M L C L C L Y V P V J G E A Q T E F Q Y F E S K G
Met

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ecor1          srf1|dcn-          pspci          mva1          eccr11|dcn-          dsav|dcn-          bsl1N1          rsa1
bsi1 foki      bsa1|dcn-          dde1|M.a1u1-          mm11 apy1|dcn1          bspcN1 mbo11          bsl1|dcn-          apo1          ac11 tsp1          tsp5091          bsp14071/bs
ecor571        a1u1          a1u1 tsp1          bstf51          bsa1 tsp5091|M.ecor1-          bsr1          tsp5091          a1u1
201 GCTCCCTGCC GAGCTGAAGT CCAATTTCAA GCTCAATGTC TTCATTCCTT CCCAGGAAT CTCACCTTAC CGCCAGTGGH AGCAGAAAAT TGTACAACT
CGAGGAGACGG CTCGACTTCA GGTAAAGAT CGAGTCACAG AAGTAAAGGA GGGTCTTAA GAGGTGATG GCGGTCACTT TCGTCTTTTA ACAATGTCGA
26 L P A E L K S J F K L S V F I P S Q E F S T Y R Q R K Q K I V Q A

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rnaI  
 maeI  
 bfaI  
 sau96I  
 ajuI  
 tseI  
 fnu4HI/bs0FI  
 apoI  
 eco0I/09I/draII  
 bbvI  
 mboII  
 tpsbuvI  
 apoI  
 mboI/ndeI|dam-|  
 ddeI|M.ajuI-|  
 bspcNI  
 trv9I  
 mseI  
 nlaI  
 kcaI  
 hpy188I  
 bspHI|dam-|  
 sau3AI  
 mboII  
 mnlI  
 301 GAGATGAG ACCTGTGATGG GCAAGCTAGAC TTTGAGAGAT TGTGTCCATT TCTCCAGAT CATGAGAGAG AGCTGAGGCT GGTGTTTAAG ATTTCAGACA  
 CCTTATTC TGGAACTAAC CGTGATCTG AACTTCTTA AACAGGTAAT AGAGGTTCTA GTACTTCTT TCGACTCCGA CCACAAATTC TAAAGCTGT  
 59 G D K D L D G D L D F E E F V H Y L Q D H E K K L R L V F K I L D K

ajwR1|dam-|

mwOI

hinfI sau3AI a1w261/hemaI

hhaI/cfoI bsaPI

thaI mboI/ndeI|dam-|

fnuDII/mvni nlaI bsmFI

bsuI|M.hhaI-| hpyCH4V

bsaI236I dpuI|dam-|

hgaI hgaI dpuI|dam+

bsmFI mlyI hpy188I

acI|drcI hinfI ecoRV

cac8I asp700 smI

apoI hpy188I

xmni tps509I

401 AAAAGATGA TGGACGATG GACCGCAGG AGATCATGCA GTCCCTGCCG GACTTGGGAG TCAAGATTTC TGAACAGCAG GCAGGAAATAA TTCTCAAGAG

TTTTCTTACT ACCTGCCTAA CTGCGGCTCC TCTAGTAAGT CAGGAGGCC CTGAACCTTC AGTTCTATAAG ACTGTCCTC GGTCTTTT AAAGCTCTC

93 K N D G R J D A Q E I M Q S L R D L G V K J S E Q Q A E K I L K S



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scrFI|dcm-}
pspGI
mvaJ
ecORI|dcm-}
dsav|dcm-}
bstNI
aJwNI|dcm-}
baskI|dcm-}
aJw26J|bsmAI sau96J|M.haeIJI-}
bceHI hpyI88IJI nIaIV apyI|dcm-}
haeIJIJ/paJI hpyCH4V bsaJI ngiAI/aspHI sau96I tseI
sau96J|M.haeIJI-} bsgI haeIJI/paJI smlI bsiHkaI hpyCH4V nIaIJI fnu4HI/bsOFI
nIaIV bcl4V bspMI bceAI bsaJI mwoI bmyI nIaIJI avaI mII aciI nIaIJI st
701 CAGGAGGTGG GCGAGGGGCC GATGTCCAGM CCGCCACGGC CCCCCTGGAC AGGCTCAAGG TGCTCATGCA EGPTCAGTGC TCCGACGCA ACAACATGGG
GTCTCCACC CCGTCCCCGG CATAGGTCTT GBACTGCCC GGGGACCTG TCCGAGTTC ACAAGTACGT CCAAGTACGG AGGGGTCTGT TGTGTACCC
193 G G G A G A V S R T C T A P I. D R J. K V I M Q V H H S P S M N N G

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tsp451
scrFI|dcn-|
pspGI
mvaI
ecoriI|dcn-|
dsav|dcn-|
bstNI
bskI|dcn-|

hpy188I taqI haeIII/paI
ddeI hpy188III nlaIV maeIII
bspcNI ttiI mnlI apyI|dcn+| acI|bsrI mnlI
msI hinfI|W.taqI-| sau96I|dcn-| |N.haeIII-| sfanI maeII/hpyCH4IV hinfI xcm

801 CATCGTTGGT GGCCTCACTC AGATGATTCG AGAAGGAGGG GCCAGGTCAC TCCTGGCGGGG CAAAGGCATC AACGTCCTCA AAATTGCCCC CGAATCGACC
GTAGCAACCA CCGAGGTGAG TCTACTTAGC TCTTCCTCC CGATCCAGTG AGACCGCCCC GTTACCCTAG TTGCAGGAGT TTTAACGGGG GCTTATGTCGG

226 I V G G F T Q W J R E G G A R S L W R G N G I N V L K I A P R S A

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scrFI|dcn-|
pspGI hpy188I
mvaI pleI styI
ecoriI|dcn-| bslI
deav|dcn-| bsaJI
bstNI mlyI sau96I
mboJ/ndeII|dcn-| ttiI avaII
dpuII|dcn-| apyI|dcn+| ddeI hinfI ppuMI sau9
tsp509I haeIII/paI dpuI|dcn+| tsp45I hinfI mnlI bssJ mnlI nlaIV nlaIV
apoJ nlaIII mkoI haeII maeIII bsmBI bspcNI hpy188III eco0109I/draII hae

901 ATCAATTCG TGGCCTATGA GCAGATCAAG CGCCTGTGTG GTAGTGACCA GGAAGCTCTG AGGATTCACG AGAGGCTGCT GGCAGGGTCC TTGBCAGGGG
TAGTTTAAGT ACCGATACT CGTCTAGTTC GCGGACACAC CATCACTGGT CCTCTGAGAC TCCTTAAGTGC TCTCCGAACA CCGTCCGAGG AACCGTCCCC

259 J K F W A Y E Q J K R L V G S D Q E T L R J H E R L V A G S L A G A

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haeIII/pal]
mscI/balI|dcm-)
eaeI|dcm-)
cfrI
scrFI|dcm-)
pspGI
nvaI xcmI
ecorII|dcm-)
dseV|dcm-)
bstNI
bssKI|dcm-)
apyJ|ocmI)
sau3AI bslI          actI
mbol/ndelI|dam-)    fnu4HI/bsoFI
dprII|dam-)          haeIII/raII
dprII|dam+)          eaeI
aIwI|dam-)           mulI   cfrI          bsmPI   nspI   sfanI   bslI   cac8I   taqI   hpyCH4V   bsmA
110) GATGCTGACC AGAAGAGGGG TGGCCGCTT CTACAAAGCC TATGTCCCA ACATGCTGGG CATCAATCCC TATGCCGCA TCGACCTTGC AGCTACGAG
CTAGGACCGG TCTCTCCCC ACCGGCGAA GATGTTCCG ATACAGGGG TGTACGACC GTAGTAGGGG ATACGGCCGT AGCTGGAACG TCAATGCTC
326 I L A R E G V A A F Y K G Y V P N M L G I I P Y A G I D L A V Y E

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mspI
hpaII
sau96I|dcn-|H.haeIII-|
    haeIII/paII    scrFI|H.hpaII-|    mspI
    scrFI|dcn-|    nciI    mroI
    pspGI    rmaI    dcaV    hpyI88I|I
    xcmI    mveI    maeI    bssKI    bspMI
    haeIII/paII    ecorII|dcn-|    sau96I    bspeI
    mscI/baII    dsav|dcn-|    nlaIV    sfanI    bsaKI    tsp45I
    eaeI    bstNI    bfaI    auaI    foki    cac8I    accII    maeIII    tseI    mboII
    cfrI    bssKI|dcn-|    ppuNI    bslI    hpyCH4V    cac8I    hinPI    mnlI    fnu4HI/bseFI
    cac8I    aIuI    apyI|dcn-|    ecoO109I/draII    hinPI    mnlI    haeII    hpaII    hphI    nlaIII    mnlI
    aIuI    cac8I    bsaOJ    bslI    hpyI88I|I    bstFSI    hhaI/cfoI    mnlI    hhaI/cfoI    bsteII    bhuI    earI/kep632I
130I AGCTGGCCAG CTACCCCTG GCCCTAGTCA GGAACCGGAT GCAAGCGCAA GCGTCTATTG AGGCGGCTCC GGAAGTGAAC ATGAGCAGCC TCTTCAGACA
TTGACCTAGTT GATGGAGGAGT GAGGATAGT GGTGGAGCTT GGTGGAGCTT GAGGAGGAGT GGTGGAGCTT GGTGGAGCTT GGTGGAGCTT GGTGGAGCTT
393 L A S Y P L A L V R T R W Q A Q A S I E G A P E V T M S S L F K H

sau96I    nlaIV    nlaIII
aveII    ecoO109I/draII    rsaI    sau96I|H.haeIII-|    aIuI    leiI
rsxII/cspI    csp6I    nlaIV    rcaI    pvuII    maeII/hpyCH4IV
cpoI    mnlI    haeIII/paII    bspI407I/bstGI    haeIII/paII    hpyI88I|I    foki    mspAII/nspBI    bsaAI
aciI    bsaOJ    sau96I|H.haeIII-|    cac8I    bspHI    bstFSI    sfanI    aIuI    accI
140I TATCTGCGG ACCGAGGGGG CCTTCGGGCT GTACAGGGGG CTGCGCCCA ACTTCATGAA GGTCAATCCA GCTGTAGCA TCACTACGT GGTTCAGAG
ATAGAGAGCC TGGCTCCCC GGAAGCCGA CATGTCCCC GACCGGGGGT TGAATACTT CCAGTAGGGT CGACACTGT AGTCGATGCA CCAAGATGCTC
426 I L R T E G A F G L Y R G L A P N F M K V I P A V S I S Y V V Y E

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scrFI|dcm-|
pspGI
mvaI
ecoriI|dcm-|
dsav|dcm-|
bstNI
bsaXI|dcm-|
bsaXI
apyI|dcmI]
bsaXI]      tsp45I
sau3AI|M.hphI]-|      aciI
mboI/ndeI|dam-|      tbaI maeII
dprI|dam-|      fnuDI/mvnI
dprI|dam+|      hpyCH4V hphI
mboI|dam-|      hseI] hseIII
eco57I hphI      cac8I bsh1236I
1501 AACCTGAAGA TCACCCCTGGG CGGTGACGGG GGGGGGCGG CCCGGCACTG GACTCGCTGA TCCTGGGCGG CAGCCTGGGG TGTGCAGCCh
TTGGACTTCTT AGTGGGACCC GCACGTCAAC GCCACTGCCC CCTTCCCGCG GGGCGGTAC CTGAGCGACT AGGACCCGCG GTCGGACCC ACACGTCGGT
459 N L K I T L G V Q S R O

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[illegible]

```

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hpaI
nael/ngom|W.haeIII|-]

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scrFI|dcn-]
alul dsai
pspGI cfrI/bsrFI btgi/
mnaI cacOI bsai
ecORI|dcn-] bsiI
dsav|dcn-] cacOI haeIII/
bstNI haeIII/paI tsel pvuII mscI/bal
bsaKI|dcn-] fnu4HI/bsofI eaeI
apyI|dcn+I bbvI mspAII/nspBJI
sfiI
hpyCH4V
hpyCH4V
pstI cacOI alul nlaIV mnlI ddeI mboII hphI hpyCH4V cfrI bce
1801 AAGACAGGA CATTTCTGC AGTGCCTGCC AATAGTAGAC TTGAGCCCTG GAGGCCGCT TAgTCTTCC ATTTCACCC TGCAGCCAGC TGTGGCCAC
TTCCTCTCC TAAAGACG TCGCGGACCG TTATCACTCG AACCTGGAC CTCGCCCA ATCAAGAGC TAACTGGCA ACTTGGCTCG ACUACCGCTG
cacOI
sau96I|W.haeIII|-]
mnlI tsel mnlI mnlI
nlaIV mnlI hpyCH4V bspI286 fnu4HI/bsofI ddeI mnlI fohI
haeIII/paI bceAI sfaKI bmyI bbvI bspCNI bserI sfiI bstf5I
1901 GGGCCCTGCC CTCTGGTCTG CCGTGCATCT CCTGTGCC TCTTGCCTGC TGCCTGTCTG CTGAGGTAG GTGGAGGAG GGTACAGCC CACATCCAC
CCGGGAGCG GAGACCAAGC GGCACGTAGA GGGACACGGG AGAACGACGG ACGACAGAC GACTCCATTG CACCTCTTC CGATGTCGG GTGTAGGCTG

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haeIII/palI
stuI|dcn-]
scrFI|dcn-]
pspGI
mvaI
ecoriI|dcn-]
taiI dsav|dcn-]
maeII/hpyCH4IV
bsaXI bstNI
pmiI bssXI|dcn-]
eco72I mliI apyI|dcn+]
bsaBI mwoI bsaJI
msp45I sfiI|dcn-]
mliI bdrPI bglI|M.haeIII-]
mliI r1aIII hpfI maeIII haeIII/paI] sfiI zhaII/hseXI
200] CCCCTCGTCC AACCCCATTA TCCATGATGA AAGGTGAAGT CAGGTGGCT CCCAGGCGTG ACTTCCCAAC CTACAGCAAT GACGCCAACT TGGCTGTGAA
GGGAGCAAGG TTAGGGTATT AGGTACTACT TTCCACATCCA GTGCACCGGA GGGTCCGGAC TGAAGGTTG GATGTCGTA CTGGGTTGA ACCGACACTT

```



	hpaI/aspHI	tsp
	mnII tspRI bspI286	haeIII/pa
	bst4C3/hpyCH411I bsiHKA3	stuI tlv9I
	tspRI nlaIII btsI bmyI nlaIII mnII	mnII meI
2301 CCCACTGTGG CATGAGGCA GTGAGCACC ATGTTTGAGG GCGAAGGCA GAGCTTGTGT GTGTCTGGG GAGGGAAGA AAGGTGTG GAGGCTTAA		
GGGTGACACC GACTCCCGT CACTCTGGT TACAACTCC CGCTTCCGT CTCGAAACA CAAAGACCC CTCCTTCTT TTTCCACAC CTCGGAAAT		
	mspI	mbdII
	mnII	bpuAI
		bbsI
	bsI4C1/hpyCH411I hpy18811I hpaII bciI hpy18811I mnII aluI	
2401 TTATGACTG TTGGGAAAG GGT17GTCC AGAAGACAA GCCGACAA TGAAGCACTT CTGTCTTCC AGAGAAAGC GAGGAGCAG GAGCTTGGCT		
AATACCTGAC AACCTTTT CCAAAACAG GTTCTCTGT CGGCTGTT ACTGCTGAA GACAGAGG TCTCTTCTG CTCCTCTGTC CTGAAACGA		
	scrI dcM-	
	pspGI	
	mvaI	sa963
	ecoriI dcM-	avaII
	p1eI	
	mnIyI	bstNI
	hinfI	bssKI dcM-
	a1wNI dcM-	bsaJI
	a1w26J/bsmAI	hinfI/acyI
	hpy188I	hgaI apyI dcM-
	ddeI	ahaII/bsaHI
	bspcNI	hpy188I bsaJI nlaIV
		bsII
		hhaI/cfoI
		fnu4HI/bscFI
		bbsVI bsmFI
		hinfI acII
		tsp45I
		meiII
		nlaI
2501 GACTGCTCAG AGTCTGTCT GAGCCTTGG GGGTCTCTGT CCAACCCAG CAGGGGCGCA GCGGAAACAG CCCACATTC CACTTGTGTC ACTGCTTGA		
CTGACGAGTC TCAAGCAAGA CTGCGGAGCC CCCAAGACA GGTGGGGTC GTCCCGCGCT CGCCTGTGTC GGGGTGTAAG GTGAACACAG TGACGAACCT		



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trv91
mseI
aseI/asnI/vepI
tsp5091
trv91
pacI
mseI  aluI
ms

260 ACCGATTTAT TTTGATTTA TTGACAGA GTTAGTCTT AACATTTT ATAGATTGT TTAATTATA GCTGTCATT TTCAGTCA TTTTATTTC
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sacI
pstI[M.HI-]
tseI
mspAII/mspBII
ecrI hpyCH4V
styI haeIII/paI
bsaJI fnu4HI/bscFI
haeIII/paI fnu4HI/bscFI
sau96I[M.haeIII-] tsp45J
nlaIV sau96I[M.haeIII-]
ecoO109I/draII hviI maeIII hpyI
nlaIII csp6I cac8I bsrI bstf5I bseRI bseRI ecoO109I/draII hviI maeIII hpyI
rsal acI bstXI foxI mui mui nlaIV sau96I[M.haeIII-]
bslI tspRI bseRI sau96I[M.haeIII-] tsp45J
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pspg1
mva1
ecor1[dcn-]
tse1 dsav[dcn-]
mwo1 bstNI
hae11/pa11 fnu4H1/bsof1
eae1 tse1 bssKI[dcn-]
bpr1/gsu1[dcn-] cfr1 fnu4H1/bsof1
tsp5091 bsr1 fnu4H1/bsof1 bbv1 apy1[dcn+] econ1
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tse1
fnu4H1/bsof1
bsr1 mml1 tll1 bsr1 bbv1 hinf1 mml1
mml1 mml1 hinf1
bsr1 nlaiv mml1 hinf1
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tspR1 ban1 bst4C1/hpyCH4111 bseR1 nlaiv mml1
hpy188? bts1 mml1 mml1 tspR1 apo1 ac11 bpm1/gsu1[dcn-] cac81 bsmf1
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haeIII/palI
eaeI
cfrI
mspI [M.haeIII]-]

tseI
mkoI      lru9I      nael/ngomI [M.haeIII]-]
{nu4HI/bsofI}      cfr101/bsrfI      hpyCH4V
bbvI      mseI      cac8I      bsrI      mmlI bstaCI/hpyCH411I      msI      mmlI      mboI]

3101 CTCCCAGCCT TCTGCTGCC TTECTTAAACA ATGCCGCCCA CTGCGGAC TCACGGTTGC ACTTCCAATC CACCAAAATG ACCGATGAG GAAATCTCA
GAGGGTCGGA AGACGACGGG AACGAATTGT TACGGCCGGT TGAACCGCTGG AGTGCCAAAG TGAAGGTAAAG GTGGTCTTAC TGGACTACTC CTTAAGAAAT

sau3A1
hpyCH4V      hpyCH4V      pleI
sfANI      mboI/ndeI (dam-)      mlyI      lru9I      tail
foKI      dnuI (dam-)      hpmI/psuI (dcm-)      mseI      maeII/hpyCH41V      ah
bstP5I      dnuI (dam-)      tsp509I      bsrI hinfI      tsp509I      tsp509I

3201 ATAGGATGCA AAGATCAATG CAAAATTGT TATATATGAA CATATTAATG GAGTGTCAA AAGCAAAAT TGAAGGTAG AAGTTGTCAAT
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tseI
{nu4HI/bsofI}
lru9I
mseI bbvI      aluI

3301 TTAAGCAGC CTCTAATTA AGTTGTTC AAGCTGAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA
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end insert

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ncII
mspi
hpaII
xmaI/bspAI      cfrI01/bsrFI
smaI              saub6I
scrFI[M.hpaII-]  xsrII/cspi
ncII              cpoi
dsav              nlaIV
fnu4HI/bsorI     aluI      bskI      hpaII      kpnI      sfcI
haeIII/peI       sstI      saI      bsaII      mroI      bsaW      sse8387I
mcrI              sacI      hincII/hindII[M.taqI-]      auaII[M.hpaII-]
eagI/xmaII/ecI>XI      accI[M.taqI-]      trv9I      hpy188II      rseI      hpyCH4V
eeei              hgiAI/aspHI[M.aluI-]      msei      bspMI      banI      pstI
cfrI              rmaI      ecII36I      auaI[M.hpaII-]      haePI      mspI      shfI
bsaEI              maeI      bsp1286[M.aluI-]      xmnI      tsp509I      ageI      csp6I
notI              bfaI      bsaHKAI      taqI      dsav      tsp509I      bsaW      hpaII      bspMI
fnu4HI/bsorI      bmyI      hpy99I      bskI      aaeI/asnI/vsPI      asp718      rsaI      aluI
acII              speI      banII[M.aluI-]      asp700      accII      acc65I      cac8I      csp6I
3401 GGGCGGCCGC CGACTAGTGA GCTCGTCGAC CCGGGAATTA ATTCCGGACC GGTACCTGCA GGCCTACCAg CTTTCCC
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> length: 3477

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aatII(GACGTC):      24
acc65I(GGTACC):     3451
accI(GTAKAC):       1192 1492 3425
accII(TCCGGA):      1368 3443

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# EXHIBIT B

[REDACTED]

User:grim

oli.out3\_6\_2000

Tue Feb 1 11:45:17 PST 2005

xe12380 / XE12380

[REDACTED]

xe12380 User:grim Job: oli.out3\_6\_2000 Date: Tue Feb 1 11:45:17 PST 2005

xe12380 User:grim Job: oli.out3\_6\_2000 Date: Tue Feb 1 11:45:17 PST 2005

xe12380 User:grim Job: oli.out3\_6\_2000 Date: Tue Feb 1 11:45:17 PST 2005

xe12380 User:grim Job: oli.out3\_6\_2000 Date: Tue Feb 1 11:45:17 PST 2005

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# EXHIBIT C

DNA #	821-823 H. Esophagus	762-763 H. Esophagus Tumor	798-799 H. Stomach	786-787 H. Stomach Tumor	792-793 H. Kidney	824-825 H. Kidney Tumor	794-795 H. Lung	776-778 H. Lung Tumor	796-797 H. Rectum	784-785 H. Rectum Tumor	833-834 H. Skin	764-766 H. Melanoma	expected size bp	source for cloning
1	58850	+	+	+	+	+	+	+	+	+	+	+		
2	58853	+	+	+	+	+	+	+	+	+	+	+		
3	58855	+	+	+	+	+	+	+	+	+	+	+		
4	59211	+	+	+	+	+	+	+	+	+	+	+		
5	59213	+	+	+	+	+	+	+	+	+	+	+		
6	59449	+	+	+	+	+	+	+	+	+	+	+		
7	59603	+	+	+	+	+	+	+	+	+	+	+		
8	59605	+	+	+	+	+	+	+	+	+	+	+		
9	59607	+	+	+	+	+	+	+	+	+	+	+		
10	59609	+	+	+	+	+	+	+	+	+	+	+		
11	59616	+	+	+	+	+	+	+	+	+	+	+		
12	59612	+	+	+	+	+	+	+	+	+	+	+		
13	59613	+	+	+	+	+	+	+	+	+	+	+		
14	59614	+	+	+	+	+	+	+	+	+	+	+		
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EXHIBIT 1

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**Front cover:** The photograph shows a rat nerve cell in culture. It is labeled ( *yellow* ) with a fluorescent antibody that stains its cell body and dendritic processes. Nerve terminals ( *green* ) from other neurons (not visible), which have made synapses on the cell, are labeled with a different antibody. (Courtesy of Olaf Mundigl and Pietro de Camilli.)

**Dedication page:** Gavin Borden, late president of Garland Publishing, weathered in during his mid-1980s climb near Mount McKinley with MBoC author Bruce Alberts and famous mountaineer guide Mugs Stump (1940–1992).

**Back cover:** The authors, in alphabetical order, crossing Abbey Road in London on their way to lunch. Much of this third edition was written in a house just around the corner. (Photograph by Richard Olivier.)

extracts. If these minor cell proteins differ among cells to the same extent as the more abundant proteins, as is commonly assumed, only a small number of protein differences (perhaps several hundred) suffice to create very large differences in cell morphology and behavior.

### A Cell Can Change the Expression of Its Genes in Response to External Signals<sup>3</sup>

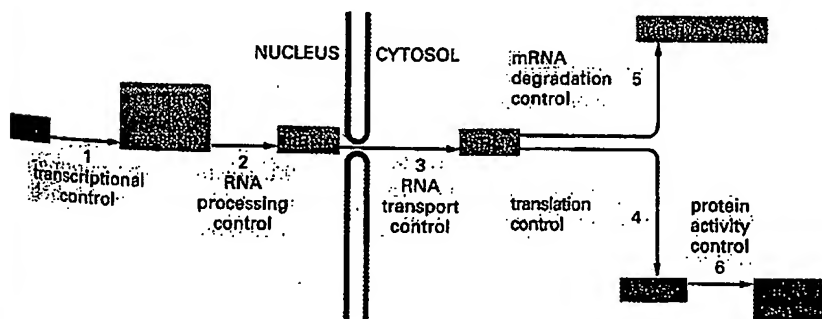
Most of the specialized cells in a multicellular organism are capable of altering their patterns of gene expression in response to extracellular cues. If a liver cell is exposed to a glucocorticoid hormone, for example, the production of several specific proteins is dramatically increased. Glucocorticoids are released during periods of starvation or intense exercise and signal the liver to increase the production of glucose from amino acids and other small molecules; the set of proteins whose production is induced includes enzymes such as tyrosine aminotransferase, which helps to convert tyrosine to glucose. When the hormone is no longer present, the production of these proteins drops to its normal level.

Other cell types respond to glucocorticoids in different ways. In fat cells, for example, the production of tyrosine aminotransferase is reduced, while some other cell types do not respond to glucocorticoids at all. These examples illustrate a general feature of cell specialization—different cell types often respond in different ways to the same extracellular signal. Underlying this specialization are features that do not change, which give each cell type its permanently distinctive character. These features reflect the persistent expression of different sets of genes.

### Gene Expression Can Be Regulated at Many of the Steps in the Pathway from DNA to RNA to Protein<sup>4</sup>

If differences between the various cell types of an organism depend on the particular genes that the cells express, at what level is the control of gene expression exercised? There are many steps in the pathway leading from DNA to protein, and all of them can in principle be regulated. Thus a cell can control the proteins it makes by (1) controlling when and how often a given gene is transcribed (**transcriptional control**), (2) controlling how the primary RNA transcript is spliced or otherwise processed (**RNA processing control**), (3) selecting which completed mRNAs in the cell nucleus are exported to the cytoplasm (**RNA transport control**), (4) selecting which mRNAs in the cytoplasm are translated by ribosomes (**translational control**), (5) selectively destabilizing certain mRNA molecules in the cytoplasm (**mRNA degradation control**), or (6) selectively activating, inactivating, or compartmentalizing specific protein molecules after they have been made (**protein activity control**) (Figure 9-2).

For most genes transcriptional controls are paramount. This makes sense because, of all the possible control points illustrated in Figure 9-2, only transcriptional control ensures that no superfluous intermediates are synthesized. In the



**Figure 9-2** Six steps at which eucaryote gene expression can be controlled. Only controls that operate at steps 1 through 5 are discussed in this chapter. The regulation of protein activity (step 6) is discussed in Chapter 5; this includes reversible activation or inactivation by protein phosphorylation as well as irreversible inactivation by proteolytic degradation.

following sections we discuss the DNA and protein components that regulate the initiation of gene transcription. We return at the end of the chapter to the other ways of regulating gene expression.

## Summary

*The genome of a cell contains in its DNA sequence the information to make many thousands of different protein and RNA molecules. A cell typically expresses only a fraction of its genes, and the different types of cells in multicellular organisms arise because different sets of genes are expressed. Moreover, cells can change the pattern of genes they express in response to changes in their environment, such as signals from other cells. Although all of the steps involved in expressing a gene can in principle be regulated, for most genes the initiation of RNA transcription is the most important point of control.*

## DNA-binding Motifs in Gene Regulatory Proteins<sup>5</sup>

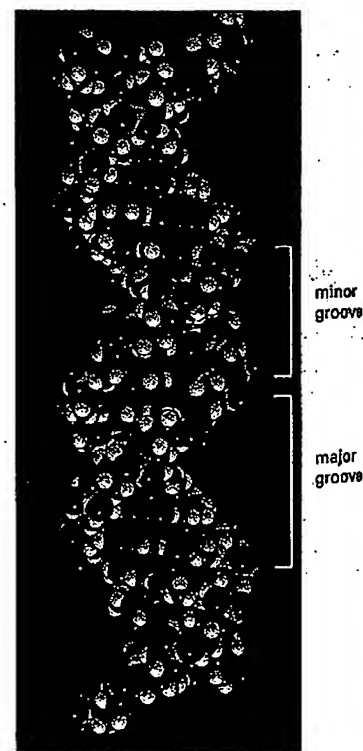
How does a cell determine which of its thousands of genes to transcribe? As discussed in Chapter 8, the transcription of each gene is controlled by a regulatory region of DNA near the site where transcription begins. Some regulatory regions are simple and act as switches that are thrown by a single signal. Other regulatory regions are complex and act as tiny microprocessors, responding to a variety of signals that they interpret and integrate to switch the neighboring gene on or off. Whether complex or simple, these switching devices consist of two fundamental types of components: (1) short stretches of DNA of defined sequence and (2) *gene regulatory proteins* that recognize and bind to them.

We begin our discussion of gene regulatory proteins by describing how these proteins were discovered.

## Gene Regulatory Proteins Were Discovered Using Bacterial Genetics<sup>6</sup>

Genetic analyses in bacteria carried out in the 1950s provided the first evidence of the existence of **gene regulatory proteins** that turn specific sets of genes on or off. One of these regulators, the *lambda repressor*, is encoded by a bacterial virus, *bacteriophage lambda*. The repressor shuts off the viral genes that code for the protein components of new virus particles and thereby enables the viral genome to remain a silent passenger in the bacterial chromosome, multiplying with the bacterium when conditions are favorable for bacterial growth (see Figure 6–80). The lambda repressor was among the first gene regulatory proteins to be characterized, and it remains one of the best understood, as we discuss later. Other bacterial regulators respond to nutritional conditions by shutting off genes encoding specific sets of metabolic enzymes when they are not needed. The *lac repressor*, for example, the first of these bacterial proteins to be recognized, turns off the production of the proteins responsible for lactose metabolism when this sugar is absent from the medium.

The first step toward understanding gene regulation was the isolation of mutant strains of bacteria and bacteriophage lambda that were unable to shut off specific sets of genes. It was proposed at the time, and later proved, that most of these mutants were deficient in proteins acting as specific repressors for these sets of genes. Because these proteins, like most gene regulatory proteins, are present in small quantities, it was difficult and time-consuming to isolate them. They were eventually purified by fractionating cell extracts on a series of standard chromatography columns (see pp. 166–169). Once isolated, the proteins were shown to bind to specific DNA sequences close to the genes that they



**Figure 9–3 Double-helical structure of DNA.** The major and minor grooves on the outside of the double helix are indicated. The atoms are colored as follows: carbon, dark blue; nitrogen, light blue; hydrogen, white; oxygen, red; phosphorus, yellow.

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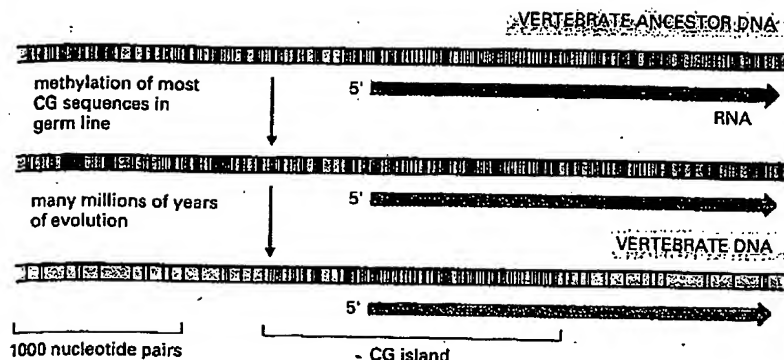


Figure 9-71 A mechanism to explain both the marked deficiency of CG sequences and the presence of CG islands in vertebrate genomes. A black line marks the location of an unmethylated CG dinucleotide in the DNA sequence, while a red line marks the location of a methylated CG dinucleotide.

## Summary

The many types of cells in animals and plants are created largely through mechanisms that cause different genes to be transcribed in different cells. Since many specialized animal cells can maintain their unique character when grown in culture, the gene regulatory mechanisms involved in creating them must be stable once established and heritable when the cell divides, endowing the cell with a memory of its developmental history. Prokaryotes and yeasts provide unusually accessible model systems in which to study gene regulatory mechanisms, some of which may be relevant to the creation of specialized cell types in higher eucaryotes. One such mechanism involves a competitive interaction between two (or more) gene regulatory proteins, each of which inhibits the synthesis of the other; this can create a flip-flop switch that switches a cell between two alternative patterns of gene expression. Direct or indirect positive feedback loops, which enable gene regulatory proteins to perpetuate their own synthesis, provide a general mechanism for cell memory.

In eucaryotes gene transcription is generally controlled by combinations of gene regulatory proteins. It is thought that each type of cell in a higher eucaryotic organism contains a specific combination of gene regulatory proteins that ensures the expression of only those genes appropriate to that type of cell. A given gene regulatory protein may be expressed in a variety of circumstances and typically is involved in the regulation of many genes.

In addition to diffusible gene regulatory proteins, inherited states of chromatin condensation are also utilized by eucaryotic cells to regulate gene expression. In vertebrates DNA methylation also plays a part, mainly as a device to reinforce decisions about gene expression that are made initially by other mechanisms.

## Posttranscriptional Controls

Although controls on the initiation of gene transcription are the predominant form of regulation for most genes, other controls can act later in the pathway from RNA to protein to modulate the amount of gene product that is made. Although these posttranscriptional controls, which operate after RNA polymerase has bound to the gene's promoter and begun RNA synthesis, are less common than transcriptional control, for many genes they are crucial. It seems that every step in gene expression that could be controlled in principle is likely to be regulated under some circumstances for some genes.

We consider the varieties of posttranscriptional regulation in temporal order, according to the sequence of events that might be experienced by an RNA molecule after its transcription has begun (Figure 9-72).

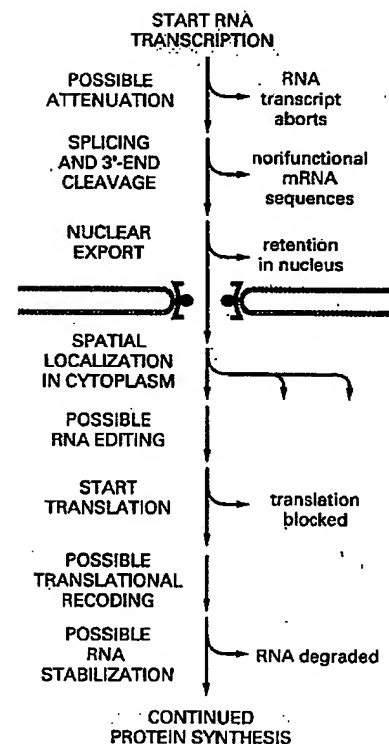


Figure 9-72 Possible post-transcriptional controls on gene expression. Only a few of these controls are likely to be used for any one gene.



## CHAPTER 29

## Regulation of transcription

Genes VI (1997) CH 29, pp. 847-848,  
Benjamin Lewin

The phenotypic differences that distinguish the various kinds of cells in a higher eukaryote are largely due to differences in the expression of genes that code for proteins, that is, those transcribed by RNA polymerase II. In principle, the expression of these genes might be regulated at any one of several stages. The concept of the "level of control" implies that gene expression is not necessarily an automatic process once it has begun. It could be regulated in a gene-specific way at any one of several sequential steps. We can distinguish (at least) five potential control points, forming the series:

Activation of gene structure  
↓  
Initiation of transcription  
↓  
Processing the transcript  
↓  
Transport to cytoplasm  
↓  
Translation of mRNA

The existence of the first step is implied by the discovery that genes may exist in either of two structural conditions. Relative to the state of most of the genome, genes are found in an "active" state in the cells in which they are expressed (see Chapter 27). The change of structure is distinct from the act of transcription, and indicates that the gene is "transcribable." This suggests that acquisition of the "active" structure must be the first step in gene expression.

Transcription of a gene in the active state is

controlled at the stage of initiation, that is, by the interaction of RNA polymerase with its promoter. This is now becoming susceptible to analysis in the *in vitro* systems (see Chapter 28). For most genes, this is a major control point; probably it is the most common level of regulation.

There is at present no evidence for control at subsequent stages of transcription in eukaryotic cells, for example, via antitermination mechanisms.

The primary transcript is modified by capping at the 5' end, and usually also by polyadenylation at the 3' end. Introns must be spliced out from the transcripts of interrupted genes. The mature RNA must be exported from the nucleus to the cytoplasm. Regulation of gene expression by selection of sequences at the level of nuclear RNA might involve any or all of these stages, but the one for which we have most evidence concerns changes in splicing; some genes are expressed by means of alternative splicing patterns whose regulation controls the type of protein product (see Chapter 30).

Finally, the translation of an mRNA in the cytoplasm can be specifically controlled. There is little evidence for the employment of this mechanism in adult somatic cells, but it does occur in some embryonic situations, as described in Chapter 7. The mechanism is presumed to involve the blocking of initiation of translation of some mRNAs by specific protein factors.

But having acknowledged that control of gene expression can occur at multiple stages, and that production of RNA cannot inevitably be equated with production of protein, it is clear

that the overwhelming majority of regulatory events occur at the initiation of transcription. Regulation of tissue-specific gene transcription lies at the heart of eukaryotic differentiation; indeed, we see examples in Chapter 38 in which proteins that regulate embryonic development prove to be transcription factors. A regulatory transcription factor serves to provide

common control of a large number of target genes, and we seek to answer two questions about this mode of regulation: what identifies the common target genes to the transcription factor, and how is the activity of the transcription factor itself regulated in response to intrinsic or extrinsic signals?

### Response elements identify genes under common regulation

The principle that emerges from characterizing groups of genes under common control is that *they share a promoter element that is recognized by a regulatory transcription factor*. An element that causes a gene to respond to such a factor is called a response element; examples are the HSE (heat shock response element), GRE (glucocorticoid response element), SRE (serum response element).

The properties of some inducible transcription factors and the elements that they recognize are summarized in Table 29.1. Response elements have the same general characteristics as upstream elements of promoters or enhancers. They contain short consensus sequences, and copies of the response elements found in different genes are closely related, but not necessarily identical. The region bound by the factor extends for a short distance on either side of

the consensus sequence. In promoters, the elements are not present at fixed distances from the startpoint, but are usually <200 bp upstream of it. The presence of a single element usually is sufficient to confer the regulatory response, but sometimes there are multiple copies.

Response elements may be located in promoters or in enhancers. Some types of elements are typically found in one rather than the other: usually an HSE is found in a promoter, while a GRE is found in an enhancer. We assume that all response elements function by the same general principle. A gene is regulated by a sequence at the promoter or enhancer that is recognized by a specific protein. The protein functions as a transcription factor needed for RNA polymerase to initiate. Active protein is available only under conditions when the gene is to be expressed; its absence means that the promoter is not activated by this particular circuit.

An example of a situation in which many genes are controlled by a single factor is provided by the heat shock response. This is common to a wide range of prokaryotes and eukaryotes and involves multiple controls of gene expression: an increase in temperature turns off transcription of some genes, turns on transcription of the heat shock genes, and causes changes in the translation of mRNAs. The control of the heat shock genes illustrates the differences between prokaryotic and eukaryotic modes of control. In bacteria, a new sigma factor is synthesized that directs RNA polymerase holoenzyme to recognize an *shpA*

Table 29.1 Inducible transcription factors bind to response elements that identify groups of promoters or enhancers subject to coordinate control.

Regulatory Agent	Module	Consensus	Factor
Heat shock	HSE	CNNGAANNTCGNG	HSTF
Glucocorticoid	GRE	TGGTACAAATGTC	Receptor
Phorbol ester	TAE	TGACTCA	AP1
Serum	SRE	CCATATTAGG	SRF

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